

CLAIMS

1. A method for modifying a biopolymer to enhance endothelial cell attachment and growth comprising coating a base biopolymer with an attachment mixture containing laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil and EGF conjugated with polycarbophil for a period of time sufficient for corneal endothelial cells to attach to and grow on said biopolymer.

2. A method of making an artificial cornea comprising:

- a) a base biopolymer;
- b) molding the biopolymer into a desired shape;
- c) coating the biopolymer with an attachment mixture comprising laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil and EGF conjugated with polycarbophil;
- d) incubating the reagent with the biopolymer at approximately 4 °C for a sufficient period of time to improve adherence of corneal endothelial cells;
- e) removing the attachment mixture; and
- f) seeding of corneal endothelial cells onto the biopolymer.

3. The method of claim 2 wherein the biopolymer is comprised of collagen IV.

4. The method of claim 2 wherein the seeding is at high density.

5. A method of making an artificial cornea comprising:

- a) a base biopolymer;
- b) molding the biopolymer into a desired shape;
- c) coating the biopolymer with a BCE-ECM coating comprising the steps of:

- 1) seeding onto the biopolymer at low density, a population of bovine corneal endothelial (BCE) cells in a culture media suitable for their growth;

- 2) allowing the BCE cells to grow to confluence;

and

- 3) aspirating the media and treating the biopolymer with ammonium hydroxide for a sufficient period of time to remove the cells;

- d) washing the biopolymer with a suitable buffer; and

- e) seeding corneal endothelial cells onto the biopolymer and growing to confluence.

6. A method of making an artificial cornea comprising:

- a) a base biopolymer;

- b) molding the biopolymer into a desired shape;
- c) coating the biopolymer with Diamond-Like Carbon using a suitable process;
- d) washing the biopolymer with a suitable buffer; and
- e) seeding corneal endothelial cells onto the biopolymer and growing to confluence.

7. A method of growing endothelial cells suitable for use in a cornea comprising:

- a) a base biopolymer;
- b) molding the biopolymer into a desired shape;
- c) coating the biopolymer an adhesion factor mixture comprising a sufficient quantity of laminin, fibronectin, RGDS, and collagen IV in a suitable biological buffer;
- d) applying the biopolymer to the corneal button; and
- e) seeding corneal endothelial cells onto the biopolymer and growing to confluence.

8. A method of growing endothelial cells suitable for use in a cornea comprising:

- a) creating a base biopolymer in contact with an adhesion factor mixture comprising a sufficient quantity of

laminin, fibronectin, RGDS, and collagen IV in a suitable biological buffer and a growth factor mixture comprising a sufficient quantity of bFGF, EGF and polycarbophil in a suitable biological buffer ;

- b) molding the biopolymer into the shape of a cornea;
- c) applying the biopolymer to the corneal button; and
- d) seeding corneal endothelial cells onto the biopolymer and growing to confluence.

9. An attachment mixture comprising laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil and EGF conjugated with polycarbophil in sufficient concentration to allow for growth of corneal endothelial cells in vitro.

10. An attachment mixture comprising:

- a) 10 µg to 500 µg/ml of fibronectin in PBS;
- b) 10 µg/ml to 500 µg/ml of laminin in PBS;
- c) 1 µg/ml to 100 µg/ml RGDS in PBS;
- d) 10 µg to 1000 µg of collagen type IV in 0.1 M acetic acid;
- e) 1 ng/ml to 500 ng/ml b-FGF in PBS; and
- f) 1 ng/ml to 500 ng/ml EGF in PBS.

11. An artificial full-thickness corneal transplant support comprising:

- a) a base biopolymer having a thickness of approximately an average cornea;

b) incorporating into the biopolymer during its synthesis an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil, EGF conjugated with polycarbophil, and heparin sulfate; and

c) molding the biopolymer into a desired shape of a cornea.

12. The composition of claim 11 wherein the biopolymer is comprised of collagen IV.

13. An artificial full-thickness corneal transplant comprising:

a) a base biopolymer having a thickness of approximately an average cornea;

b) incorporating into the biopolymer during its synthesis an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil, EGF conjugated with polycarbophil, and heparin sulfate;

c) molding the biopolymer into the shape of a cornea;

d) seeding HCEC onto the biopolymer and growing to confluence.

14. An artificial half-thickness corneal transplant support comprising:

a) a base biopolymer having a thickness of approximately one half the thickness of an average cornea;

b) incorporating into the biopolymer during its synthesis an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil, EGF conjugated with polycarbophil, and heparin sulfate; and

c) molding the biopolymer into the shape of a cornea.

15. An artificial half-thickness corneal transplant comprising:

a) a base biopolymer having a thickness of approximately one half the thickness of an average cornea;

b) incorporating into the biopolymer during its synthesis an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil, EGF conjugated with polycarbophil, and heparin sulfate;

c) molding the biopolymer into the shape of a cornea;

d) seeding HCEC onto the biopolymer and growing to confluence.

16. The artificial cornea of claim 15 wherein the biopolymer is collagen IV.

17. The artificial cornea of claim 1 wherein the biopolymer is non-swelling in the presence of culture media.

18. A method of repairing a damaged cornea comprising the steps of:

a) obtaining an artificial full-thickness cornea which has been seeded with HCEC and allowed to grow a sufficient period of time so that the HCEC are confluent;

b) implanting the artificial full-thickness cornea of step a onto a damaged cornea;

c) securing said cornea by surgical or other means.

19. A method of repairing a damaged cornea comprising the steps of:

a) obtaining an artificial full-thickness cornea;

b) overlaying said corneal surface with a biopolymer having confluent HCEC on it;

c) implanting the artificial full-thickness cornea of step a onto a damaged cornea;

d) securing said cornea by surgical or other means.

20. A method of repairing a damaged cornea comprising the steps of:

a) obtaining an artificial half-thickness cornea which has been seeded with HCEC and allowed to grow a sufficient period of time so that the HCEC are confluent;

b) implanting the artificial half-thickness cornea of step a onto a damaged cornea;

c) securing said cornea by surgical or other means.

21. A method of repairing a damaged cornea comprising the steps of:

a) obtaining an artificial half-thickness cornea;

b) overlaying said corneal surface with a biopolymer having confluent HCEC on it;

c) implanting the artificial half-thickness cornea of step a onto a damaged cornea;

d) securing said cornea by surgical or other means.

22. A method for making retinal pigment epithelial (RPE) cells suitable for transplantation into a retina comprising the steps of:

a) obtaining a biopolymer having a top and a bottom surface and having a thickness between about 10 to 100  $\mu$ m in thickness;

b) placing said biopolymer in a medium suitable for the



growth of RPE cells in vitro;

c) seeding RPE cells onto the top surface of said biopolymer sheet at a certain density and allowing the RPE cells to grow to confluence; and

d) removing said sheet and cutting to a desired size.

23. The method of claim 22 wherein the biopolymer is biodegradable.

24. The method of claim 22 wherein the biopolymer is embedded or has incorporated into it during its synthesis an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophil, EGF conjugated with polycarbophil, and heparin sulfate.

25. A composition comprising retinal pigment epithelial (RPE) cells suitable for transplantation into a retina made using the method of claim 22.

26. A method of repairing a retina in vivo comprising the steps of:

a) identifying the damaged area of a retina to be repaired;

b) aspirating remaining RPE cells from the damaged retinal area;

- c) obtaining retinal pigment epithelial (RPE) cells suitable for transplantation into a retina made by the method of claims 1, 2 or 3;
- d) aspirating the biopolymer with the RPE on its top side into a cannula or other suitable aspiration means;
- e) injecting an air bubble of suitable size into the damaged area of a retina to be repaired;
- f) positioning the biopolymer with the RPE on its top side onto the damaged area with the cells on its top side; and
- g) aspirating the air bubble in the retinal space.